Effect of Concentration of Coeluting Internal Standards on Dynamic Range in Ion Trap Quantitative Analyses

Application Note

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Introduction

Early ion trap mass spectrometer designs were not well suited to quantitative analyses. Differing concentrations of coeluters such as deuterated internal standards sharply reduced linearity and made quantitation difficult. Modern ion trap designs with external ionization sources such as the atmospheric pressure ionization (API) sources commonly used for LC/MS anayses have largely erased this problem. They are linear over three orders of magnitude and provide excellent quantiation results. This application note demonstrates this linearity even in the presence of vastly excessive concentrations of a coeluting internal standard.

This note also demonstrates some advantages of ion trap technology over triple-quadrupole instruments for quantitative applications in high-throughput environments. These advantages include the ability to acquire full scan MS/MS spectra for more structural information with no loss of sensitivity, the ability to select quantitation ions post acquisition without reanalyzing samples, and the ability to sum multiple quantitation ions for increased sensitivity.

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Experimental

All experiments were performed using an Agilent 1100 Series LC/MSD Trap ion trap mass spectrometer coupled to an Agilent 1100 Series HPLC system. The system was fitted with an electrospray ionization (ESI) source and operated in the positive ion mode.

The sample, terbutaline (MW 225.28), and the deuterated analog terbutaline-d_9 used as an internal standard, were supplied by AstraZeneca (Lund, Sweden). Terbutaline is the active component in Bricanyl®, a prescription drug used in the treatment of asthma. The substance acts as a bronchodilator by relaxing the smooth muscle in the bronchi and blood vessels.

A quantitative assay was developed with the instrument operated in the full scan MS/MS mode. The quantitation ions were selected post acquisition and were summed from the following transitions of terbutaline and the terbutaline-d_9 analog:

Terbutaline:
MS/MS of *m/z* 226 –> *m/z* 152 + *m/z* 170

Terbutaline-d_9:
MS/MS of *m/z* 235 –> *m/z* 152 + *m/z* 153 + *m/z* 171

Calibration curves were generated over the range of 3.3 pg to 3.4 ng for terbutaline using the deuterated analog (terbutaline-d_9) as an internal standard. In order to demonstrate that increasing levels of coeluting compounds have no adverse effects on the performance of the LC/MSD Trap, the amount of internal standard was increased 10, 100, and 1,000 fold and quantitation assays were run at each level.

ANALYSIS METHOD:

LC/MS/MS

Column:	Zorbax SB C_{18} 3 × 150 mm
Flow rate:	0.4 ml/min
Injection volume:	50 µl
Mobile phase:	A = 1% Acetic acid (Aq)
Mobile phase:	B = MeOH
Gradient:	Isocratic at 15% B

MS Conditions

Ionization Mode:	Positive ESI
Drying gas flow:	9 l/min
Nebulizer:	35 psig
Drying gas temperature:	350°C
Skim 1:	25.0 V
Cap Exit Offset:	70 V
Averages:	2
ICC:	On
Max Accumulation Time:	300 ms
Target:	12000
Isolation width:	1.5 *m/z*
Fragmentation Amp:	0.77 V

Results and Discussion

Figure 1 shows the extracted ion chromatograms of terbutaline and terbutaline-d_9, including their corresponding full scan MS/MS spectra.

The full scan MS/MS spectrum of terbutaline reveals three major product ions at *m/z* 208.0, 169.9, and 152.0. The ion at *m/z* 208.0 corresponds to the loss of a water molecule. It was not used for quantitation due to its lack of specificity. The ion at *m/z* 169.9 corresponds to the loss of isobutene and the ion at *m/z* 152.0 corresponds to isobutene loss followed by the loss of water.

The full scan MS/MS spectrum of the terbutaline-d_9 internal standard reveals major product ions at *m/z* 217.1, 170.9, 153.1, and 152.1. The ion at *m/z* 217.1 corresponds to the loss of water. It was not used for quantitation. The ion at *m/z* 170.9 corresponds to the loss of isobutene-d_8. The ions at *m/z* 153.1 and 152.1 correspond to isobutene-d_8 loss followed by the loss of water. Two different ions result because sometimes a deuterated water molecule is lost.

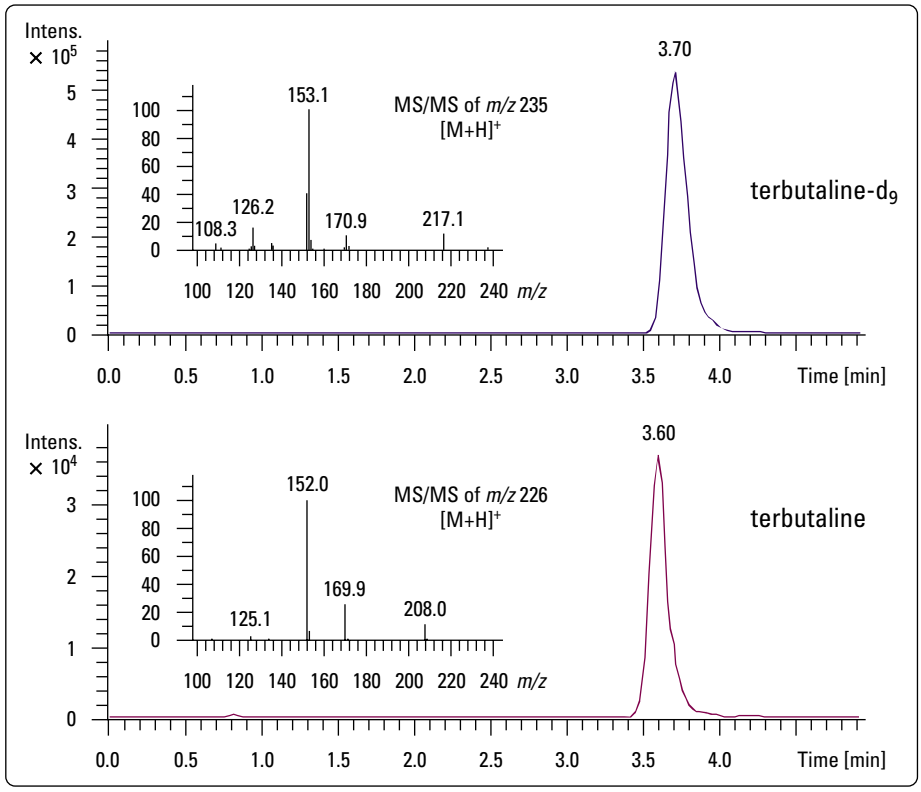

Figure 1. Extracted ion chromatograms of terbutaline and its deuterated analog, terbutaline-d_9, as internal standard; full scan MS/MS spectra of terbutaline and terbutaline-d_9 ISTD (inset)

The calibration plot in Figure 2 demonstrates linearity and dynamic range for the LC/MSD Trap exceeding three orders of magnitude with 0.395 ng of coeluting internal standard injected on column.

Figures 3–5 show the calibration curves generated for the 10, 100 and 1,000 fold excesses of coeluting internal standard, respectively. The LC/MSD Trap demonstrates good linearity and dynamic range for the terbutaline quantitation assay with no negative effects, even in the presence of a massive amount of coeluting internal standard.

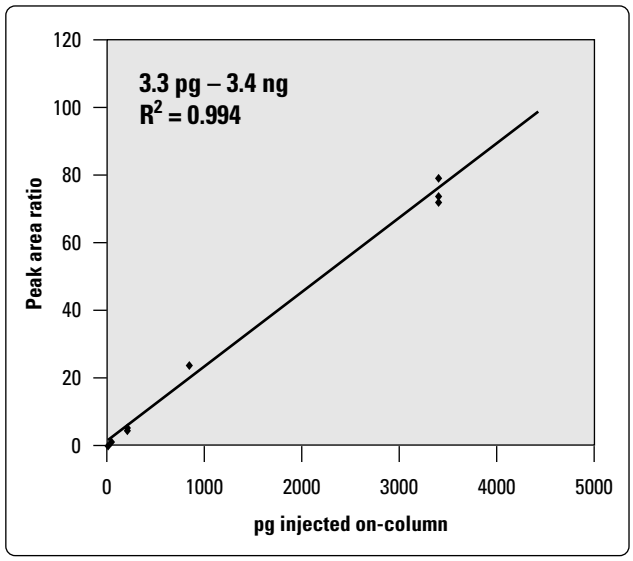

Figure 2. Calibration curve for the quantitation of the terbuatline standard using 0.395 ng internal standard

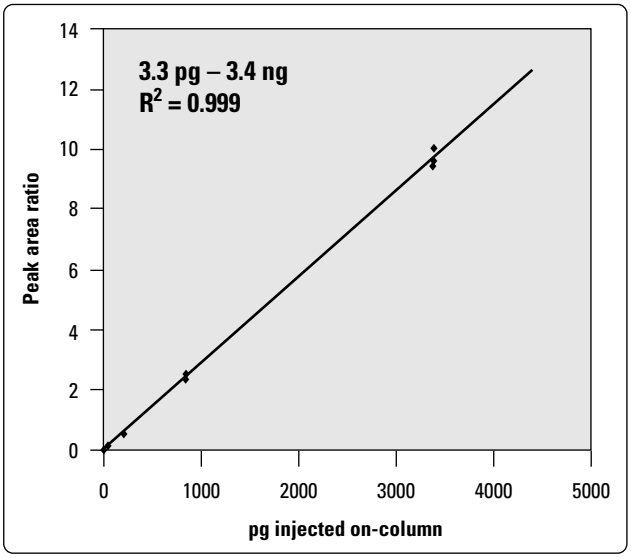

Figure 3. Calibration curve for the quantitation of the terbutaline standard using 3.95 ng internal standard

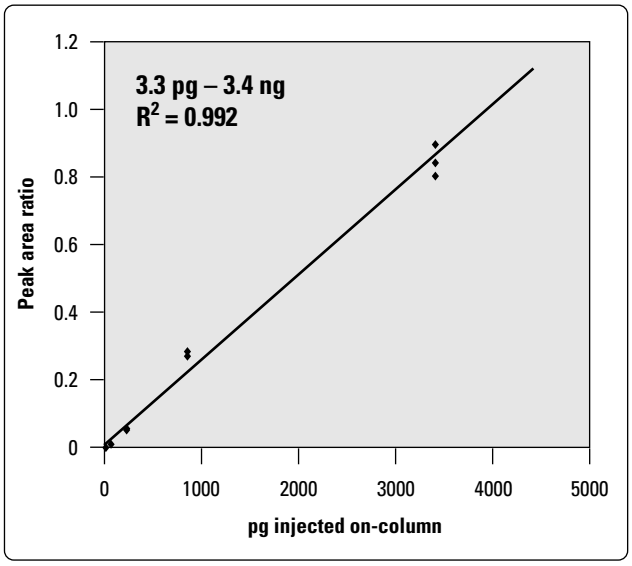

Figure 4. Calibration curve for the quantitation of the terbutaline standard using 39.5 ng internal standard

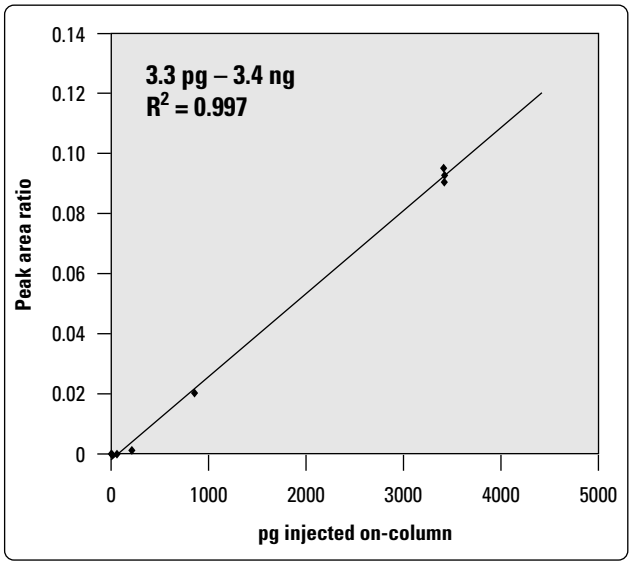

Figure 5. Calibration curve for the quantitation of the terbutaline standard using 395 ng internal standard

Conclusions

The LC/MSD Trap demonstrates linearity over the three orders of magnitude for quantitative assays. The presence of increasing amounts of coeluting internal standard has no effect on the analytical linearity or instrument performance. Quantitative assays performed in the full scan MS/MS mode provide the added benefit of structural information, resulting in more reliable compound identification and quantitation. Furthermore, the use of full scan MS/MS assays on the LC/MSD Trap permits significantly more rapid method development because the choice of quantitation ions can be made post acquisition, saving time and precluding the need to reanalyze samples.

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